

Amendments to the Specification:

Please amend the specification as follows¹:

Please replace the Title with the following amended Title:

SYSTEMS AND METHODS FOR ENLARGING THE DIAMETER OF AN ARTERY OR VEIN IN A HUMAN SUBJECT OPENING OBSTRUCTED BIOLOGICAL CONDUITS

Please replace paragraph [0001]² with the following amended paragraph:

This application is a continuation of ~~depending application(s)~~ application number 09/669,051. ~~The nonprovisional application designated above, namely application U.S. Application No. 09/669,051, filed September 24, 2000, which claims the benefit of U.S. Provisional Application(s) No(s). 60/155,938, filed September 24, 1999. This application claims the benefit of U.S. Provisional Application Ser. No. 60/155938, filed Sep. September 24, 1999, each of which is incorporated by reference herein in its entirety.~~

Please replace paragraph [0008] with the following amended paragraph:

The extracellular matrix, which holds a tissue together, is composed primarily of collagen, the major fibrous component of animal extracellular connective tissue (Krane, *J. Investigative Dermatology* (1982) 79:83s-86s; Shingleton, *Biochem. Cell Biol.*, (1996) 74:759-75). The collagen molecule has a base unit of three strands[[,]] of repeating amino acids coiled into a triple helix. These triple helix coils are then woven into a right-handed cable. As the collagen matures, cross-links form between the chains and the collagen becomes progressively more insoluble and resistant to lysis. When properly formed, collagen has a greater tensile strength than steel. Not surprisingly, when the body builds new tissue collagen provides the

¹ For the convenience of the Examiner, applicant refers to the numbered paragraphs as they appear in United States Patent Application Publication US 2004/0265294 A1, attached hereto as Appendix A.

² The first two sentences of the text being amended, although not reflected in the published application, were inserted by preliminary amendment dated June 24, 2003. See Transmittal Letter at § 17.B.

extracellular structural framework such that the deposition of hard collagen in the lesion can result in duct obstruction.

Please replace paragraph [0009] with the following amended paragraph:

Benign biliary stricture results in obstruction of the flow of bile from the liver and can result in jaundice and hepatic dysfunction. If untreated, biliary obstruction can result in hepatic failure and death. ~~Biliary~~ Biliary strictures can form after duct injury during cholecystectomy. They can also form at biliary anastomoses after liver transplantation and other biliary reconstructive surgeries (Vitale, *Am. J. Surgery* (1996) 171:553-7; Lilliemoe, *Annals of Surgery* (1997) 225).

Please replace paragraph [0010] with the following amended paragraph:

Historically, benign biliary stricture has been treated surgically by removing the diseased duct segment and reconnecting the duct end-to-end, or connecting the duct to the bowel via a hepaticojejunostomy loop (Lilliemoe, *Annals of Surgery* (1997) 225). These long and difficult surgeries have significant morbidity and mortality due to bleeding, infection, biliary leak, and recurrent biliary obstruction at the anastomosis. Post-operative recovery takes weeks to months. More recently, minimally invasive treatments such as percutaneous balloon dilation have been utilized, yielding good initial biliary patency ~~surgeries~~ results (Vitale, *Am. J. Surgery* (1996) 171:553-7, Lilliemoe, *Annals of Surgery* (1997) 2250). However, balloon dilation causes a localized injury, inducing a healing response that often results in restenosis (Pauletto, *Clinical Science*, (1994) 87:467-79). Long-term stenting at the common bile duct with flexible biliary drainage catheters is another minimally invasive alternative to surgery (Vitale, *Am. J. Surgery* (1996) 171:553-7). However, these indwelling biliary drainage catheters often become infected, or clogged with debris, and must be changed frequently. At present, long-term treatment of biliary stricture remains a difficult clinical problem.

Please replace paragraph [0011] with the following amended paragraph:

Patients with chronic, end-stage renal failure may require replacement of their kidney function in order to survive. In the United States, long-term hemodialysis is the most common treatment method for end stage chronic renal failure ~~in the U.S.~~ In 1993, more ~~[[-]]~~ than 130,000 patients underwent long term hemodialysis (Gaylord, *J. Vascular and Interventional Radiology* (1993) 4:103-7), ~~More 4:103-7);~~ more than 80% of these patients implement hemodialysis through the use of a synthetic arteriovenous graft (Windus, *Am. J. Kidney Diseases* (1993) 21:457-71). In a majority of these patients, the graft consists of a 6 mm Gore-Tex tube that is surgically implanted between an artery and a vein, usually in the forearm or upper arm. This high flow conduit can then be accessed with needles for hemodialysis sessions.

Please replace paragraph [0017] with the following amended paragraph:

It thus would be desirable to provide new methods to relieve obstructions blocking flow through ~~biological~~ biological conduits.

Please replace paragraph [0019] with the following amended paragraph:

Methods and systems of the invention can be applied to a variety of specific therapies. For example, methods of the invention include treatment of ~~biliary~~ biliary stricture with the use of exogenous collagenase, elastase or other agent, whereby an enzyme composition comprising collagenase, elastase or other agent is directly administered to or into (such as by catheter injection) the wall of the lesion or other obstruction. The ~~enzyme(s)~~ enzyme(s) dissolves the collagen and/or elastin in the extracellular matrix, resulting in the solubilization of fibrous tissue from the duct wall near the lumen, and a return of duct flow or opening.

Please replace paragraph [0021] with the following amended paragraph:

Preferably, the therapeutic agent is delivered ~~proximately~~ proximately to a targeted site, e.g. by injection, catheter ~~delivery~~ delivery or the like.

Please replace paragraph [0023] with the following amended paragraph:

Specifically preferred therapeutic agents for use in the methods and systems of the invention include proteases and other enzymes e.g. a collagenase ~~e.g. such as~~ Clostridial collagenase, a proteolytic enzyme that dissolves collagen, and/or an elastase such as a pancreatic elastase, a ~~proteolytic~~ proteolytic enzyme that dissolves elastin. Preferred delivery of collagenase and other therapeutic agents of the invention include directly injecting the agent into the target lesion or other obstruction. Preferably, a homogeneous distribution of a therapeutic enzyme or enzyme mixture is administered to a target site with a drug delivery catheter. The therapeutic agent can then dissolve the key extracellular collagen components necessary to solubilize the obstructing tissue from the vessel wall near the lumen.

Please replace paragraph [0026] with the following amended paragraph:

In another aspect of the invention, treatment compositions and treatment kits are provided. More particularly, ~~treatment~~ treatment compositions of the invention preferably contain one or more ~~enzymatic~~ enzymatic agents such as collagenase preferably admixed with a pharmaceutically acceptable carrier. Such compositions can be suitably packaged in ~~conjunction~~ conjunction with an appropriate delivery tool such as an injection syringe or a delivery catheter. The delivery device and/or treatment solution are preferably packaged in sterile condition. The delivery device and treatment composition can be packaged separately or in combination, more ~~typically~~ typically in combination. The delivery device preferably is adapted for in situ, preferably ~~localized~~ localized, delivery of the therapeutic agent directly into the targeted ~~biological~~ biological conduit obstruction.

Please replace paragraph [0027] with the following amended paragraph:

~~Typical~~ Typical subjects for treatment in accordance with the invention include mammals, particularly primates, especially humans. Other subjects may be treated in accordance with the invention such as domesticated animals, e.g. pets such as dogs, cats and the like, and

horses and livestock animals such as cattle, pigs, sheep and the like. Subjects that may be ~~treating~~ treated in accordance with the invention include those mammals suffering from or susceptible to biliary stricture including benign biliary stricture, stenosis of hemodialysis graft, intimal ~~hyperlasia~~, hyperplasia, and/or coronary obstruction, and the like. As discussed above, methods of the invention may be administered as a pre-treatment protocol before ~~other~~ another therapeutic regime such as a balloon angioplasty; during the course of another therapeutic regime, e.g. where a therapeutic composition of the invention is administered during the course of an angioplasty or other procedure; or after another treatment regime, e.g. where a therapeutic composition of the invention is administered after an angioplasty or ~~administration~~ administration of other therapeutic agents.

Please replace paragraph [0035] with the following amended paragraph:

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of introducing a therapeutic agent that is ~~capable~~ capable of degrading an extracellular matrix ~~components~~ component to thereby facilitate the reopening of a constricted biological conduit. In particular, the invention provides for introduction to an obstructed ~~biological~~ biological conduit of a therapeutic agent that degrades collagen and/or elastin. The present invention further provides methods of ~~dilating~~ dilating a biological conduit by introducing a therapeutic agent into a biological ~~conduit~~, conduit, preferably an isolated segment of the conduit.

Please replace paragraph [0042] with the following amended paragraph:

Preferred therapeutic agents for use in accordance with the invention include those that exhibit digestion activity in such a standard *in vitro* tissue digestion assay at least about 10 percent greater relative to a control, more preferably at least about 20% greater digestion activity relative to a control; still more preferably at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%

or 100% greater digestion activity relative to a control ~~in such a standard in vitro tissue digestion assay.~~

Please replace paragraph [0043] with the following amended paragraph:

Appropriate therapeutic agents can comprise at least one and frequently several enzymes such that the therapeutic agent is capable of degrading both significant matrix components of tissue obstruction. Particularly preferable therapeutic agents will comprise either a collagenase or elastase or both. Specifically preferred are therapeutic agents comprising a highly purified, injectable collagenase preparation ~~suchs such as that~~ produced from cultures of ~~Clostridia histolyticum~~ *Clostridium histolyticum* by BioSpecifics Technologies Corporation (Lynbrook, NY). This enzyme preparation is composed of two similar but distinct collagenases. The Clostridial collagenases cleave all forms of collagen at multiple sites along the helix, rapidly converting insoluble collagen fibrils to small, soluble peptides. Also preferable are therapeutic agents comprising elastase, particularly pancreatic elastase, an enzyme capable of degrading elastin. Trypsin inhibitors also can be suitably employed as the therapeutic agent in the methods of the invention.

Please replace paragraph [0048] with the following amended paragraph:

In a preferred aspect of the invention, a therapeutic agent comprising at least one enzyme capable of degrading elastin, collagen or both is delivered to the targeted obstruction site with a catheter. Preferred catheters are capable of directly localizing a therapeutic agent directly into the extracellular matrix of the obstruction. Particularly preferable catheters are ~~able~~ capable of delivering accurate doses of the therapeutic agent with an even distribution over the entire obstructed area of the conduct. One particularly preferred example of a catheter for use in the method of the present invention is the Infiltrator® catheter produced by InterVentional Technologies Corporation (IVT) (San Diego, CA), which delivers a precisely controlled dosage of a drug directly into a selected segment of vessel wall (Figure 1) (Reimers, *J. Invasive*

Cardiology (1998) 10:323-331; Barath, ~~Catherterization~~ Catheterization and Cardiovascular Diagnosis (1997) 41:333-41; Woessner, *Biochem. Cell Biol.* (1996) 74: 777-84). Using this preferred catheter a therapeutic agent can be delivered at low pressure via a series of miniaturized injector ports mounted on the balloon surface. When the positioning balloon is inflated, the injector ports extend and enter the vessel wall over the 360° surface of a 15 mm segment of vessel. Each injector port is less than 0.0035 inch in size. Drug delivery can be performed in less than 10 seconds, with microliter precision and minimal immediate drug washout. The injected drug is delivered homogeneously in the wall of the vessel or duct (Figure 2). The triple lumen design provides independent channels for guidewire advancement, balloon inflation and drug delivery. Trauma associated with injector port penetration is minimal and the long-term histologic effects are negligible (Woessner, *Biochem. Cell Biol.* (1996) 74: 777-84). In addition, the device has been engineered such that the injector ports are recessed while maneuvering in the vessel. Additionally, the Infiltrator® catheter is capable of balloon inflation with sufficient force for angioplasty applications. The excellent control of drug delivery observed with Infiltrator® can be significant since preferred therapeutic agents of the present invention potentially can degrade collagen and/or elastin in nearly all forms of tissue in a non-specific manner.

Please replace paragraph [0049] with the following amended paragraph:

In yet another embodiment of the present invention, a therapeutic dose is employed which will restore conduit flow while maintaining conduit wall integrity. Several parameters need to be defined to maximize method efficiency, including the amount of enzyme to be delivered, and the volume of enzyme solution to be injected so that the reopening of the conduit occurs with a single dose protocol. Ideally repeat or multiple dosing is reserved only for patients who have an incomplete response to the initial injection.

Please replace paragraph [0056] with the following amended paragraph:

The invention also includes ~~prophalytic-type~~ prophylactic-type treatment, e.g. methods to ~~dilate~~ dilate a biological conduit whereby the increased conduit diameter obviates the potential of obstruction formation with a conduit. Temporary and partial ~~degradation~~ degradation of the elastin component of a conduit wall reduces the elasticity of the ~~conduit~~ conduit, thereby facilitating modifications of the size and shape of the conduit. Introducing a dose of therapeutic agent in accordance with the invention into the lumen of an isolated conduit or some section thereof results in complete or partial diffusion of the therapeutic agent into the wall of the isolated conduit during a specified period of time. Subsequent pressurization of the treated ~~region~~ region, either while the region is still isolated or after removing the means of ~~isolation~~ isolation, increases the lumen diameter by dilation. Regeneration of the conduit elastin framework results in a conduit with a larger lumen diameter ~~and~~ without compromising the structural integrity.

Please replace paragraph [0057] with the following amended paragraph:

Arteriovenous hemodialysis grafts are frequently placed in the arm of the patient such that blood can be withdrawn and purified blood returned through the graft. Frequently the lumenal diameter of the venous outflow is smaller than the graft lumenal diameter. Development of a stenosis due to intimal hyperplasia can further reduce the lumenal diameter of the venous outflow such that an insufficient volume of blood passes through the venous outflow. To prevent intimal hyperplasia and stenosis formation, dilating the venous outflow vein using the above described method of partially degrading the elastin component of the vascular wall downstream of the site of graft implantation such that the lumenal diameter of the venous outflow is similar to or larger than the diameter of the interposed loop graft reduces the likelihood of forming ~~of~~ a stenosis due to intimal hyperplasia. Venous ~~dilation~~ dilation can be ~~performed~~ performed either before or after interposing a graft between the artery and vein.

Please replace paragraph [0060] with the following amended paragraph:

The rate of tissue digestion, which is composed mostly of collagen, by a mixture of collagenase and elastase, proteolytic enzymes with activity ~~respectfully~~ respectively against collagen and elastin, was determined. Trypsin inhibitor was added to negate the effect of any residual trypsin activity. Briefly, fresh pig tendon was excised, trimmed, washed, blotted dry and weighed. Individual tendon pieces were suspended in 3.58 mg/ml HEPES buffer at neutral pH and various concentrations of enzymes were added. Iodinated radiographic contrast was added in various concentrations to some of the enzyme solutions. The tissue digestion was carried out in a water bath at 37°C. At various time points, the tendon pieces were removed from the enzyme solution, washed, blotted dry and weighed. Each time point was derived from the average of three samples. The effect of enzyme concentration on tissue digestion rates was studied. As expected, increasing the concentration of enzymes in vitro increased the rate of tissue digestion (Figure 3). Buffer alone had no effect on the tissue. Extrapolating digestion rates in vitro to an in vivo situation has proven difficult. For Dupuytren's contractures, the effective dose for transecting fibrous cords in vitro was 500 ABC units. However, the effective in vivo dose was 10,000 ABC units.

Please replace paragraph [0062] with the following amended paragraph:

Example 2. Determining dose ~~dependant~~ dependent in vitro activity of a therapeutic agent including collagenase, elastase, and a trypsin inhibitor.

The effect of enzyme concentration on tissue digestion rates was studied (Figure 3). The "1x" tissue sample was treated with collagenase 156 Mandel units/ml + elastase 0.125 mg/ml + trypsin inhibitor 0.38 0.38 mg/mg, The "2x" sample was treated with collagenase 312 Mandel units/ml + ~~elastase~~ elastase 0.25 mg/ml + trypsin inhibitor 0.76 mg/ml. The "5x" sample was treated with collagenase 780 Mandel units/ml + ~~elastase~~ elastase 0.625 mg/ml + trypsin inhibitor 1.9 mg/ml. All digestion volumes were 0.5 ml. Increasing the concentration of enzymes in vitro

increased the rate of tissue digestion (Figure 3). Buffer alone had no effect on the tissue. An effective in vivo dose was found to be 10,000 ABC units.

Please replace paragraph [0063] with the following amended paragraph:

Example 3. Determining the effect of iodinated radiographic contrast material on tissue digestion rates to facilitate monitoring enzyme delivery prior to injection of a therapeutic agent comprising a contrast material into a patient.

The “35% Omnipaque” tissue sample was treated with collagenase 156 Mandel units/ml + elastase 0.125 mg/ml + 0.38 trypsin inhibitor with 35% ~~Omnipaque~~ Omnipaque 350 contrast (volume:volume). The “5% Omnipaque” sample was treated with collagenase 312 Mandel units/ml + ~~elastase~~ elastase 0.25 mg/ml + 0.76 trypsin inhibitor with 5% Omnipaque 350 (volume:volume). The “1% Omnipaque” sample was treated with collagenase 312 Mandel units/ml + elastase 0.25 mg/ml + 0.76 trypsin inhibitor with 1% Omnipaque 350. All digestion volumes were 0.5 ml. These results demonstrate that Omnipaque 350 iodinated contrast material inhibits enzyme activity at radiographically visible (35%) concentrations, but not at lower (1-5%) concentrations (Figure 4). Similar results were observed with Hypaque 60 contrast.

Please replace paragraph [0066] with the following amended paragraph:

Example 5: ~~Relieve~~ Relief of strictures in the common bile duct of a patient.

A large dog was used as the patient such that under general anesthesia a cholecystostomy tract was created and the gallbladder was “tacked” to the abdominal wall with retention sutures. A cholangiogram was performed with Hypaque-60, using a marker catheter, in order to define the anatomy. Then, a flexible catheter with a bipolar electrode tip was constructed as previously described (Becker, *Radiology* (1988) 167:63-8). This catheter was inserted through the gallbladder (Figure 5) and positioned with its “hot” tip (arrow) in the distal common bile duct such that the catheter was pulled back and the treatment was repeated until a 1.0 cm length of duct was injured (Figure 6). Immediately after delivering the current there was a mild-moderate amount of smooth narrowing of the treated segment of duct (arrow), possibly due to spasm or edema. A pigtail nephrostomy drainage catheter was then inserted through the fresh cholecystostomy tract into the gallbladder. The distal end was closed with an IV cap and buried in the subcutaneous tissue. The surgical wounds were then closed in a two-layer fashion.

Please replace paragraph [0067] with the following amended paragraph:

After 7 days, a follow-up cholangiogram was performed to evaluate the thermally induced stenosis. A 20 gauge needle was used to ~~percutaneous~~ percutaneously access ~~then the~~ the drainage catheter through the IV cap. A cholangiogram was performed demonstrating moderate-marked dilation of the biliary tree (Figure 1). There was a high-grade stricture of the mid common bile duct, where the thermal injury had been made.

Please replace paragraph [0068] with the following amended paragraph:

Strictures are created in five large dogs using the methods described above and in Example 4. In addition, an objective measurement of biliary patency (the Whitaker study) is made of the common bile duct, both before and after making a stricture. The Whitaker study is performed by injecting normal saline through a catheter positioned in the common bile duct.

Flow rates are increased and pressure measurements are taken ~~with~~ until a peak pressure of 40 mmHg is reached.

Please replace paragraph [0072] with the following amended paragraph:

After treatment with collagenase, a final cholangiogram is taken after 1 week (Figure 2). At this time, the animal is sacrificed and the extrahepatic biliary tree harvested. Histologic assessments are made of the bile duct proximal to the treated lesion, the mid portion of the treated lesion (Figure 5), the treated lesion edge, and the duct distal to the lesion. Assessments of 1) duct morphology, 2) cell type and number, 3) the extent and appearance of the extracellular matrix, and 4) extent of epithelialization were made. Figure 5 is a histology image of a common bile duct stricture after treatment. The arrows denote the outer limit of collagen breakdown. The histological examination of the treated common bile duct stricture demonstrates as a circumferential lysis of collagen at the treatment site, while sparing damage to the normal duct, arteries and veins.

Please replace paragraph [0073] with the following amended paragraph:

Example 6: ~~Relieve~~ Relief of stenosis due to intimal hyperplasia of a synthetic hemodialysis graft.

Standard, untapered 5 mm diameter polytetrafluoroethylene (PFTE) loop grafts were interposed between the femoral artery and the femoral vein in the hind limbs of 25-35 kg dogs, as described previously (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). An end-to-end configuration had been selected to facilitate optimal positioning of the catheter drug delivery balloon during treatment of a stenosis. Standard, cut-film angiography is performed one week after surgery to assess the arterial inflow, the artery-graft anastomosis, the vein-graft anastomosis, and the venous outflow. After this, routine physical examination of the grafts will be carried out to screen for patency. Twenty weeks after surgery, standard, cut-film angiography is performed to assess the luminal diameter of the grafts and their venous outflow.

At this time, a stenosis due to intimal hyperplasia is seen in the venous outflow with an associated pressure gradient (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). Then, using the first animal, the therapy delivery catheter is deployed within a graft and 5000 ABC units of collagenase in 0.5 ml is infiltrated into the wall of the lesion at the venous outflow. The catheter is flushed and the contralateral lesion receives 1 ml of saline, delivered in an identical manner. Nearly all collagenase activity is extinguished after 1-2 days such that the grafts are re-examined with angiography after 3 days. Repeat measurements of luminal diameter and invasive pressure measurements across the lesion are also taken. The animals are sacrificed and the grafts excised, pressure-fixed, and examined histologically. Assessments are made of the distal graft, the venous anastomosis, the mid-portion of the treated lesion, the lesion edge, and the normal vein downstream from the graft. Additional assessments of 1) cell type, morphology and number, 2) extent of extracellular matrix, 3) overall adventitial, medial, and intimal thickness, 4) extent of intimal hyperplasia, and 5) extent of endothelialization are made.

Please replace paragraph [0074] with the following amended paragraph:

Four dogs are used for a controlled study of collagenase treatment. Bilateral grafts are created as described previously and standard, cut-film ~~angiography~~ angiography is performed one week after surgery to access the arterial inflow, the artery-graft anastomosis, the vein-graft anastomosis, and the venous outflow. After this, routine physical examination of the grafts ~~are~~ is carried out to screen for patency. Then, twenty weeks after surgery, standard, cut-film ~~angiography~~ angiography is performed to assess the luminal diameter of the grafts and their venous outflow. An obvious stenosis due to intimal hyperplasia is usually seen in the venous outflow with an associated pressure gradient (Trerotola, *J. Vascular and Interventional Radiology* (1995) 6:387-96). The Infiltrator catheter is then deployed within the lesion and the selected dose of collagenase is infiltrated into the wall of the lesion. The contralateral, control graft is treated in an identical manner, except that saline ~~will be~~ is delivered instead of collagenase. Three days after treatment, the grafts are restudied with ~~an~~ angiography and

invasive pressure measurements to determine the acute effects of collagenase treatment.

Changes in luminal diameter and pressure gradients are calculated for both the collagenase-treated group and the saline-treated group and ten days after collagenase treatment, the grafts are studied a final time. The animals ~~will be~~ are sacrificed and the grafts ~~will be~~ are excised, pressure-fixed, and examined histologically, as described above.